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EARLY GENITAL STIMULATION OF RATS LOWERS LIMBIC SEIZURE LATENCIES FOR FEMALES BUT INCREASES LATENCIES FOR MALES [1]

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Summary.—The genitals of male and female rats were tactilely stimulated (glass rod) for 10 successive days beginning on postnatal Days 5, 15, or 35; handled but nonstimulated litter mates served as the reference groups. Limbic seizures were induced by a single systemic injection of lithium and pilocarpine when the rats were adults. The genitally stimulated female rats displayed a lower seizure threshold (as inferred from shorter seizure-onset times) relative to their cage mates. The single largest effect occurred for those females which had been stimulated after the vagina had opened (postnatal 35–45 days).

The hypothesis that early sexual abuse contributes to the development of complex partial epileptic seizures (Schenk & Bear, 1981; Benson, Miller, & Signer, 1986): (1) directly, due to persistent stress-related glucocorticosteroid effects upon neuronal development or (2) indirectly, due to a kindling-like process that subsequently alters behavior and personality, has been assumed or proposed by several authors (Goodwin, 1993). However, with the exception of the consequences of physical distress and pain associated with sexual penetration for young females, there is no clear neuroanatomical rationale by which stimulation of the vagina, by itself, should necessarily evoke aberrant permanent changes in brain micromorphology.

Classic neuroscience has indicated that stimulation of all sensory systems evokes specific changes within their target regions of the thalamus and cerebral cortices. Either insufficient or excessive stimulation can produce neuronal necrosis and transneuronal degeneration or alternative proliferation of dendrite arborization (e.g., Greenough, Volkmar, & Juraska, 1973). Whereas the consequences of repeated, enhanced sensory stimulation upon cortical thickness was greater within the occipital region of male rats, the same stimulation produced primary changes in female rats within the somesthetic areas (Diamond, 1976) which represent the body image. In principle, without the intervening variable of social value judgement, the genitals in general and the lining of the vaginal wall in particular are simply biological sensing areas represented within brain space. Like other sensory systems, excessive

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stimulation of these body loci should alter the dendritic patterns within the respective projection sites of the cortices.

The localization of genital projections within brain space is likely to be multifocal. For human beings, the male external genitals are primarily represented along the medial surface of the paracentral lobule (Carpenter & Sutin, 1983) immediately below the representation of the feet. Although a similar representation within this region of the paracentral lobule should be evident for the clitoris, direct measurements have not been evident. On the other hand, the epithelial lining of the deep vagina and the uterus are internal organs and should be represented beneath the lateral sulcus (Sylvian Fissure) within the parietal operculum. In some species the cortical projection sites for the deep vaginal lining may involve portions of the adjacent insular and orbital frontal cortices (Newman, 1974). Involvement of the amygdaloid region has been suggested by the female-specific experience of sexual arousal during ictal discharges within this limbic structure (Remillard, Andermann, Testa, Gloor, Aube, Martin, Feindel, Guberman, & Simpson, 1983).

We have been pursuing the relationship between complex partial epileptic seizures and partial epileptic-like seizure signs upon behavior and changes in behavioral patterns, i.e., personality (Persinger & Makarec, 1993). If excessive early genital stimulation preferentially affects the limbic system and results in micromorphological changes that encourage later psychogenic or clinical seizures in humans, then comparable changes should occur, in principle, within the rat brain. Considering the marked concordance in receptor characteristics within the limbic and thalamic regions for the rat brain and the human brain (Mendelsohn & Paxinos, 1991) as well as the similarities of basic cell functions involved with neuroplasticity (Haas, Greene, Heimrich, & Xie, 1988), species similarity would be expected.

We predicted that the synaptic modification due to early vaginal stimulation would be more evident in adult female rats than in adult male rats and should be exhibited as faster seizure-onset times. We reasoned that female rats would be more vulnerable because (1) early stimulation preferentially affects their somesthetic cortical areas (Diamond, 1976), (2) the medial nucleus of the female amygdala is more than twice the size of the same structure within the male brain (Malsbury & McKay, 1989), (3) this electrically labile region is critical to the initiation and propagation of limbic seizures, and (4) autogenic lesions from lithium/pilocarpine-induced limbic seizures have suggested a high probability pathway between the somatosensory parietal cortices and this region of the amygdala (Persinger, Bureau, Kostakos, Peredery, & Falter, 1993).

Method

To test these hypotheses, a total of 12 litters (Wistar) from 120 day-old

Wistar females were culled to seven to nine pups each within 24 hr. of birth. Four litters were randomly assigned to one of three groups which were treated on either postnatal Days 5 through 15, postnatal Days 15 through 25, or postnatal Days 35 through 45. All litters were weaned on postnatal Day 22 and were maintained in cages of same sex groups (3/cage). The treatment was standard for each group. Half of the numbers of the males and the females for each litter served as handled (for 5 min.) reference groups. The other rats from each litter received genital stimulation from a small glass stirring rod for five consecutive minutes. Identification of the rats was maintained by a small nontoxic colour marker. The specific colour was alternated between stimulated and handled rats for the different litters. Except for the 35- through 45-day-old female rats, whose vaginal apertures had opened, the genital stimulation was exclusively along the clitoral/penile urethral region.

In two separate episodes, when the rats were between 80 and 120 days of age, they were injected subcutaneously with 3 mEq/kg of lithium and then 4 hr. later with 30 mg/kg of pilocarpine (subcutaneous). The time (in minutes) for the display of forepaw clonus, rearing and falling (the limbic motor seizure) was recorded (Persinger, Makarec, & Bradley, 1988) for each rat. The two experimenters who observed and recorded the rats' behaviors were not familiar with the characteristics of the hypothesis or the treatment condition of the animals. All statistical analyses involved SPSS software that was executed on a VAX 4000 computer.

Results and Discussion

A three-way analysis of variance of the seizure-onset time as a function of sex (male, female), treatment (handled only, genitally stimulated), and time of treatment (early, middle, late) yielded a statistically significant sex difference ($F_{1,60}=21.64$, $p<.001$; $eta=0.48$) and an interaction of sex by treatment ($F_{1,60}=6.87$, $p=.01$). There were no other statistically significant main effects or interactions. *Post hoc* (Tukey's test set at $p<.05$) analyses of the two-way interaction (four groups; 18 rats/group) showed that genitally stimulated females exhibited shorter seizure-onset times ($M=31.5$, $SD=9.0$ min.) than their handled-only, same-gender cage mates ($M=37.4$, $SD=11.5$ min.), while the genitally stimulated males exhibited longer seizure-onset times ($M=27.1$, $SD=8.4$ min.) than their same-sex cage mates ($M=22.4$, $SD=5.1$ min.).

It may be relevant that the three-way interaction between sex, stimulation, and time of handling approached statistical significance ($F_{2,60}=2.75$, $.05<p<.10$). *Post hoc* analysis of this interaction (12 groups; 6/group) showed that the largest difference occurred between the females which had been stimulated after the vagina had opened ($M=26.8$, $SD=9.6$ min.) and

the females which were stimulated during the first 10 days ($M = 40.3$, $SD = 10.2$ min.). The likelihood of a body-weight artifact was minimal. In addition to the expected greater weight of the male rats relative to the female rats ($F_{1,60} = 296.26$, $p < .001$; $eta = 0.88$), there was a significant time-of-treatment effect ($F_{2,60} = 11.59$, $p < 001$; $eta = 0.24$). This was due in large part to the lighter weights of all the male ($M = 414 \pm 65$ gm) and female rats (253 ± 28 gm) regardless of stimulation condition, treated between 35 and 45 days of age compared to those groups treated during postnatal Days 5 to 15 (508 ± 44 gm; 278 ± 37 gm) and postnatal Days 15 to 25 (497 ± 63 gm; 291 ± 25 gm).

The results confirm the hypothesis that males and females may respond differentially to early genital stimulation. That the synaptic alterations which are assumed to have followed the stimulation resulted in permanent lowering of limbic seizure threshold was suggested by the faster seizure-onset times for the genitally stimulated females relative to their male litter mates which received this stimulation. The greatest discrepancy between the treatments occurred between the females whose vaginal linings had been penetrated compared with the handled-only reference group.

Although the seizure-onset times for all of the vaginally stimulated females were only about 6 minutes faster than their controls, this discrepancy could reflect substantial microstructural alteration when one considers the time frame (1 msec. to 10 msec.) within which synaptic activity occurs. This interval, which would be equivalent to a process which proceeded at least 10,000 times faster in the vaginally stimulated rats, suggests a marked difference in the functional organization of the neuropathways which propagate the paroxysmal discharges which begin in the piriform/amygdaloid region and then spread to motor centers (wherein the consequences of the discharges become behaviorally obvious). By comparison, treatment with estradiol (10 mg/kg) on prenatal Day 19 (Whetstone, 1994) or three days of successive treatment with the glucocorticoid suppressor dexamethasone produced shifts of only about 10 minutes (Richards, Persinger, Harrigan, & Whetstone, submitted).

In this context, the difference of 13 min. in seizure-onset time between the vaginally stimulated and control females which were treated during the peripubertal period (35–45 days) may be biologically significant and deserves further study. However, we cannot exclude the possibility that this form of genital stimulation altered the blood-brain exchange of either lithium or pilocarpine. We also cannot exclude an alternative hypothesis that micromorphological changes due to the vaginal stimulation may have occurred within the ventrobasal complex of the thalamus (Berkley, Guilbaud, Benoist, & Gautron, 1993). The possibility that the estrus phase at the time of the induction of the seizures may have confounded the results is not

likely considering the absence of statistical significance in seizure-onset times between groups of female rats which were within one of the four phases (Persinger, *et al.*, 1988).

To our knowledge this is the first study in which experimental stimulation of genitals was repeated during periods of ontogenetic development that would be compatible to human sexual abuse and then the possibility of long-term changes in limbic electrical lability was assessed. Sexual dimorphism of the male/female human brain (Remillard, *et al.*, 1983) and the inherently greater access to body representation within other brain structures such as the insula and orbital frontal region, may help explain the greater reported effects of protracted sexual penetration upon consequent behavior. Since, however, the threshold for pain and nonnoxious tactile/proprioceptive stimulation is frequently exceeded in cases of human sexual abuse, the differential contributions of frank nociception and sensory stimulation may be more academic than practical. From this perspective the use of a glass rod, which would have been associated with significant mechanical (nonfluid) resistance, can be argued to simulate closely the somatosensory correlates of extreme genital stimulation.

Several tests of these hypotheses could be pursued. First, the insular region is involved with conditioned taste aversion. Early stimulation of the vagina or of the uterus should alter either the threshold for tastes or the recovery curve for the aversion to tastes. Secondly, excessive stimulation of the vaginal/uterine representation within the insular-orbitofrontal region should be reflected in more frequent periods, particularly during sensitization by hormonal cycles, of disinhibition of prefrontal tasks. Third, because the insula is also associated with the chronicity of cardiac activity, genitally stimulated females should exhibit a greater risk of cardiovascular anomalies and sudden cardiac death when the protective effects of circulating estrogens are reduced. Fourth, direct access to the insula following frequent premature stimulation of the vagina and cervix during ontogenetic windows of higher order dendritic arborization would also directly affect its direct connections with the regions of the (central nucleus of) amygdala which modulate the ventromedial hypothalamus. Consequently, the early stimulated females should exhibit, as adult animals, similar but less dramatic characteristics of the ventromedial hypothalamic syndrome such as insidious weight gain and alterations in palatability of foods. Options two and four in combination might constitute a precursor for bulimia.

If these data may be generalizable to humans, then early excessive deep vaginal stimulation could evoke synaptic changes within the sexual dimorphic regions of the amygdala and adjacent limbic areas. If early painful sexual abuse facilitates limbic lability and contributes to the wide spectrum of complex partial epileptic-like seizures (e.g., see Devinsky, Putnam, Graf-

man, Bromfield, & Theodore, 1989), then the concordance of pseudoseizures, dissociative states, and temporal lobe epilepsy may have a rational neuroanatomical explanation. The extent to which routine and unintentional stimulation, which would occur during rhythmic mechanical impacts (bathing, restrictive clothing around the pelvis, horseback riding, or accidents), could alter this lability is also not clear.

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